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The chemistry of mycophenolic acid – synthesis and modifications towards desired biological activity

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Abstract

Mycophenolic acid (MPA) is a basis for the immunosuppressive drugs used in clinic against rejection in solid organs transplantations. Since its physiological activity is very promising, numerous studies have been performed to establish mechanism of action, structure – activity relationship (SAR), synthesis of MPA derivatives to improve or extent its clinical use to anticancer one, especially. The reported methods for preparation of MPA analogues have been achieved by semi-synthetic approaches or total synthesis and accomplished by *in vitro* or / and *in vivo* evaluations. In this review we would like to bring together chemical aspects of these compounds and their implementations within biological activity, their synthesis and structural modifications referred to the structure-activity relationship (SAR).

Key words:

mycophenolic acid, MPA, MPA analogues.

Introduction

Mycophenolic acid (4*E*)-6-(4-Hydroxy-6-methoxy-7-methyl-3-oxo-5-phthalanyl)-4-methyl-4-hexenoic acid (**1)** Fig. (**1**) is a type of immunosuppressive drug widely used for prophylaxis and treatment of organ rejection in transplantation [1-4].

The mechanism of its action is based on inhibition of inosine-5'-monophosphate dehydrogenaze (IMPDH). The enzymes involved in nucleotides biosynthesis are crucial for supporting cell proliferation. In general, there exist two pathways of nucleotide biosynthesis – *de novo* (when purine or pyrimidine ring has to be assembled) and salvage pathway (when already existed nucleobases are recycled).

IMPDH (inosine-5'-monophosphate dehydrogenaze) is the enzyme which catalyzes the rate determining step in guanine nucleotide biosynthesis *de novo*. This NAD^+ dependent reaction involves conversion of IMP (inosine-5'-monophosphate) to XMP (xanthine-5'-monophosphate) [5]. Mycophenolic acid is active towards two human isoforms of the enzyme: IMPDH I (expressed in normal cells) and IMPDH II (observed at high levels in neoplastic cells) [1, 6].

In commerce are applied: ester - mycophenolate mofetil (**2**) (MMF; CellCept®, Roche AG) and sodium salt – mycophenolate sodium (**3**) (MPS; Myfortic®, Novartis Pharma AG) [7-9] both in combination with corticosteroids and calcineurin inhibitors (cyclosporine A, tacrolimus) [10-13]. The FDA approved MMF as an immunosuppressive agent for organ transplant in 1995 [1]. This compound possesses also interesting antiviral, antibacterial, antifungal and antipsoriatic properties [11, 14-16]. The anticancer activity of MPA have been also examined, however its glucuronide metabolite MPAG (**4**) occurred to be not active [1, 17, 18].

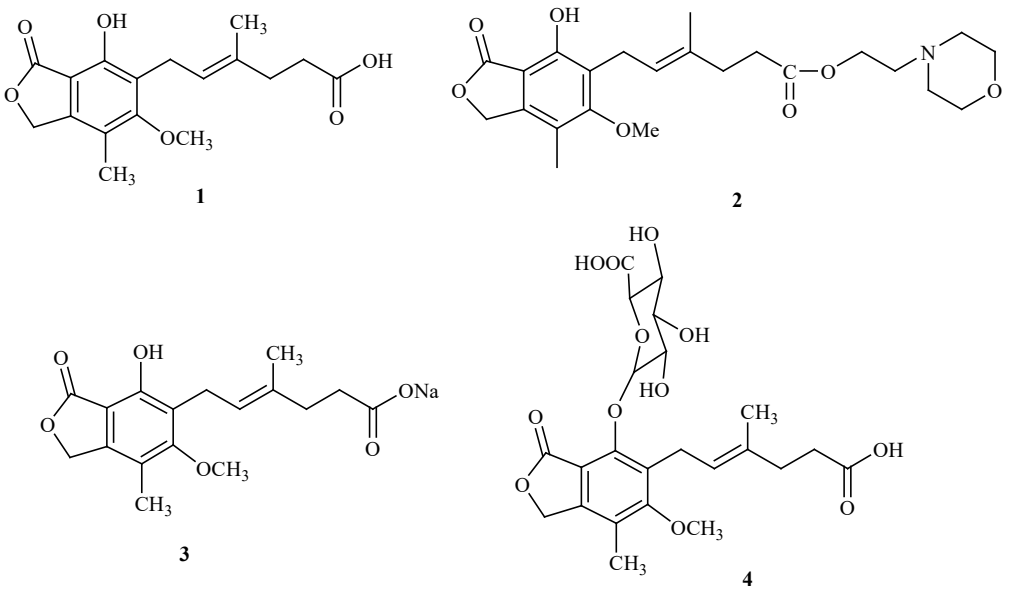


Fig. (1). Mycophenolic acid **1** and its derivatives **2-4**

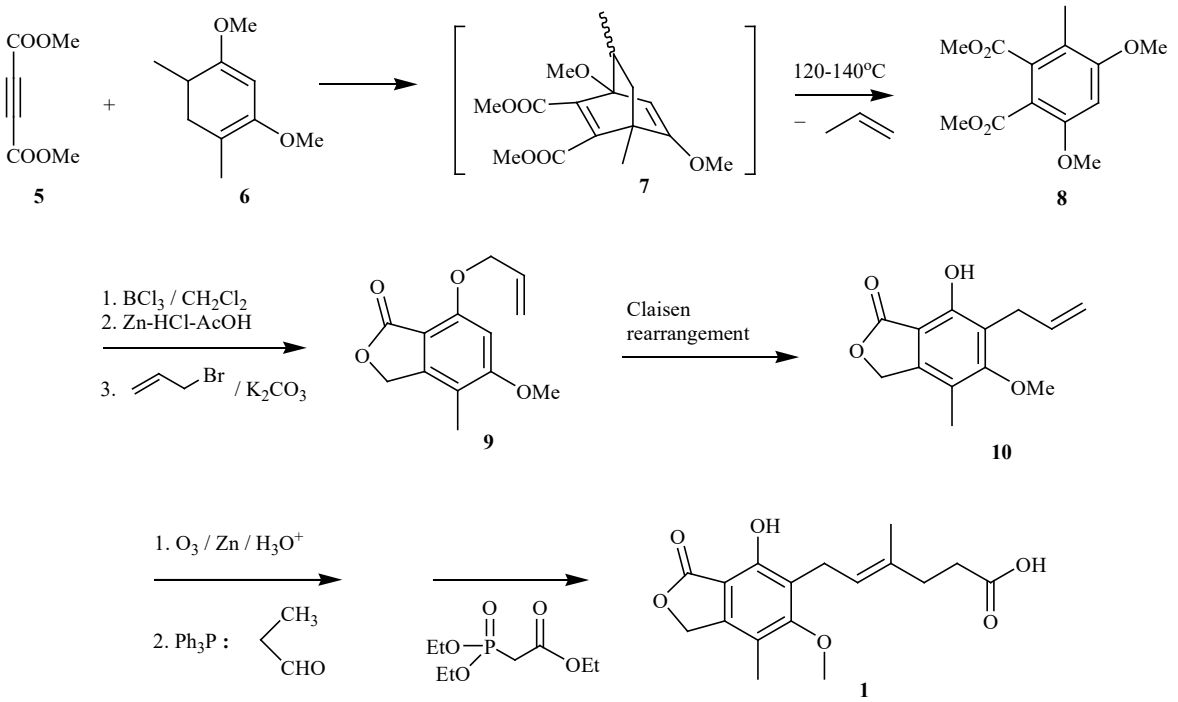
Synthesis of MPA

The structure of mycophenolic acid (**1**) includes six-substituted benzene ring fused with lactone moiety. Other functional groups are: hydroxyl, methoxyl, methyl and alkyl side chain bearing six carbons, double bond in *trans* conformation and free carboxylic group. This compound was discovered by Gosio in 1896 [1], and its structure was established by Clutterbuck [19] and Raistrick [20]. The first synthesis was published by Birch and Wright [21, 22]. Further modifications and other methods aimed at improving yield, reducing number of stages or using distillation or crystallization instead of chromatography purification to scale-up purpose [23-33]. However, synthesis of MPA (**1**) from commercially available starting materials is still rather time-consuming. Mycophenolic acid (**1**) is also produced from *Penicillium brevicompactum* [1, 32]. The widespread method is a solid-state fermentation, which enables to reach higher fermentation productivity, lower catabolic repression, low water demand, lower sterility demand in comparison with other biotechnological processes [34]. On the other hand, chemical synthesis provides various structure alteration, in some

cases difficult to achieve by simple modifications of MPA molecule. Those target molecules are challenging for organic chemists looking for relevant synthetic strategies.

Analogues of MPA were also produced by microbial transformations, however their biological activity examinations were not reported [1, 35, 36].

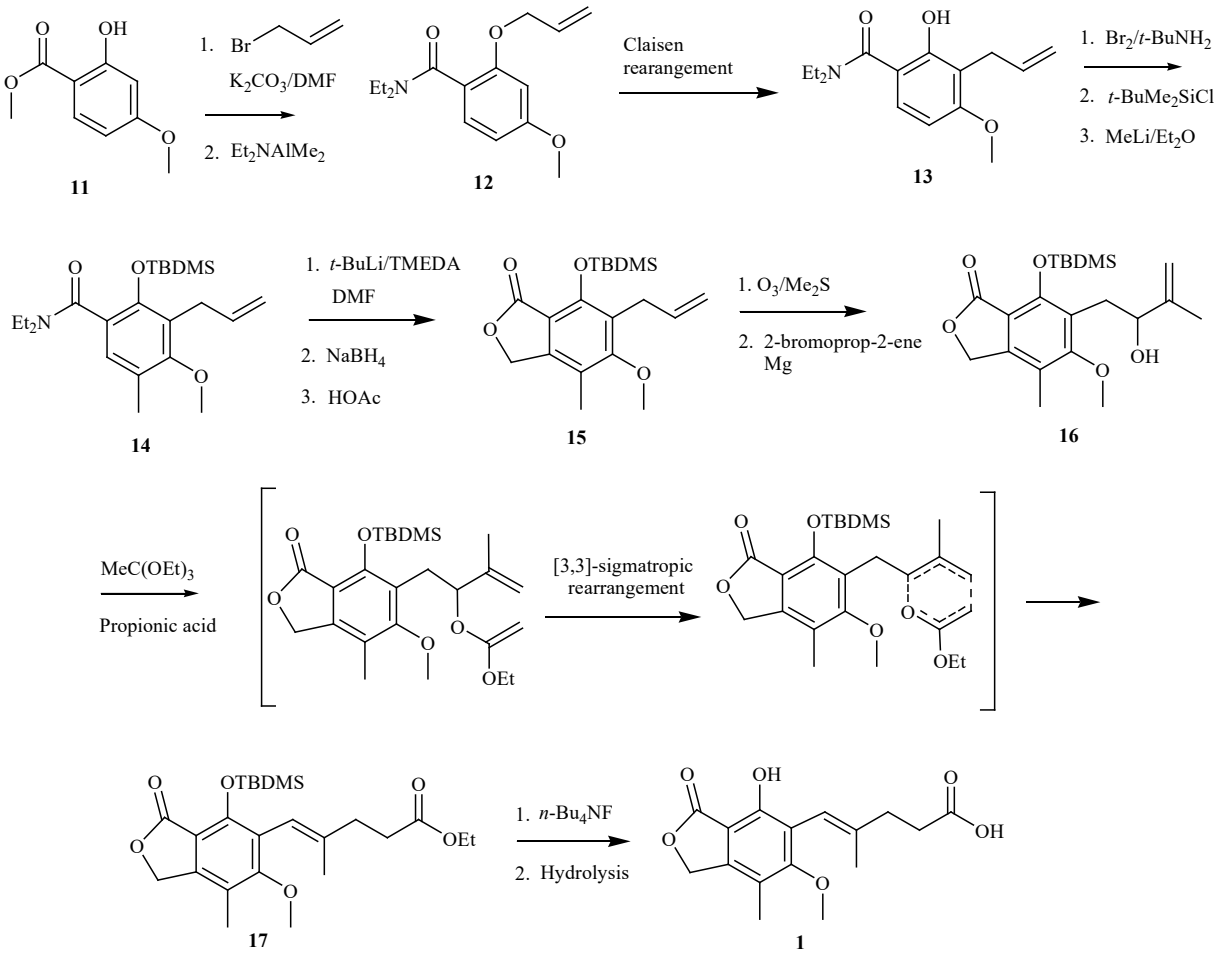
The key step in the first synthesis of MPA described by Birch and Wright (Scheme **1**) was Alder –Rickert reaction of 1,3-dimetoxy-4,6-dimethyl-1,3-cyklohexadiene (**5**) with dimethyl acetylenedicarboxylate DMAD (**6**). The bicyclic Diels-Alder adduct (**7**) was not isolated, but heated to achieve five-substituted benzene (**8**) via propylene elimination. The substituents were modified towards allyl ether (**9**) which underwent Claisen rearrangement to phenol (**10**). Then, transformations of introduced side chain gave mycophenolic acid (**1**) [21].



Scheme 1. Synthesis of mycophenolic acid **1** reported by Birch and Wright [21]

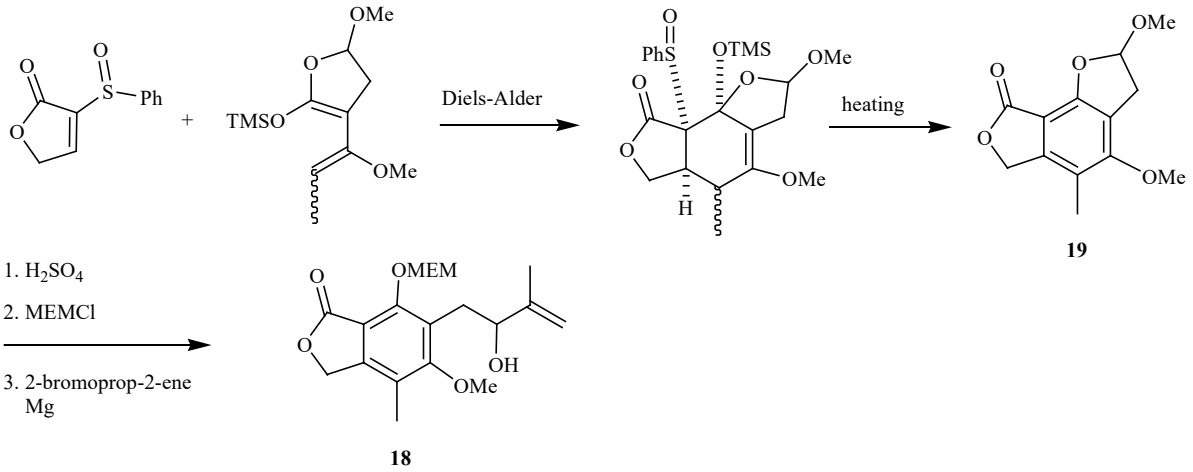
Patterson described synthetic pathway (Scheme **2**) from commercially available methyl 2-hydroxy-4-metoxybenzoate [23, 24]. The convenience of this laboratory method is a reduction

of chromatographic separations and scaled-up to several grams of MPA. The starting material (**11**) holds three substituents in the aromatic ring. *N,N*-Diethyl-4-methoxy-2-(prop-2-enyloxy)benzamide (**12**) gave phenol (**13**) in Claisen rearrangement, which was brominated and methylated at 6 position, after protection of phenol group. Then, *N,N*-Diethyl-2-[(*tert*-butyldimethylsilyl)oxy]-4-methoxy-5-methyl-3-(prop-3-enyl)benzamide (**14**) was formylated, followed by reduction of aldehyde group with sodium borohydride to alcohol and lactonization in the presence of acetic acid. 1,3-Dihydro-4-[(*tert*-butyldimethylsilyl)oxy]-6-methoxy-7-methyl-3-oxo-5-(prop-2-enyl)isobenzofuran (**15**) underwent oxidation to respective aldehyde, which was converted to allylic alcohol (**16**) in the reaction with 2-propenyl magnesium bromide. The allylic alcohol (**16**) was a substrate in the Johnson-Claisen rearrangement with triethyl orthoacetate in propionic acid to produce *trans*-alkene (**17**). Finally, deprotection of phenol group and hydrolysis of ester gave mycophenolic acid **1**.



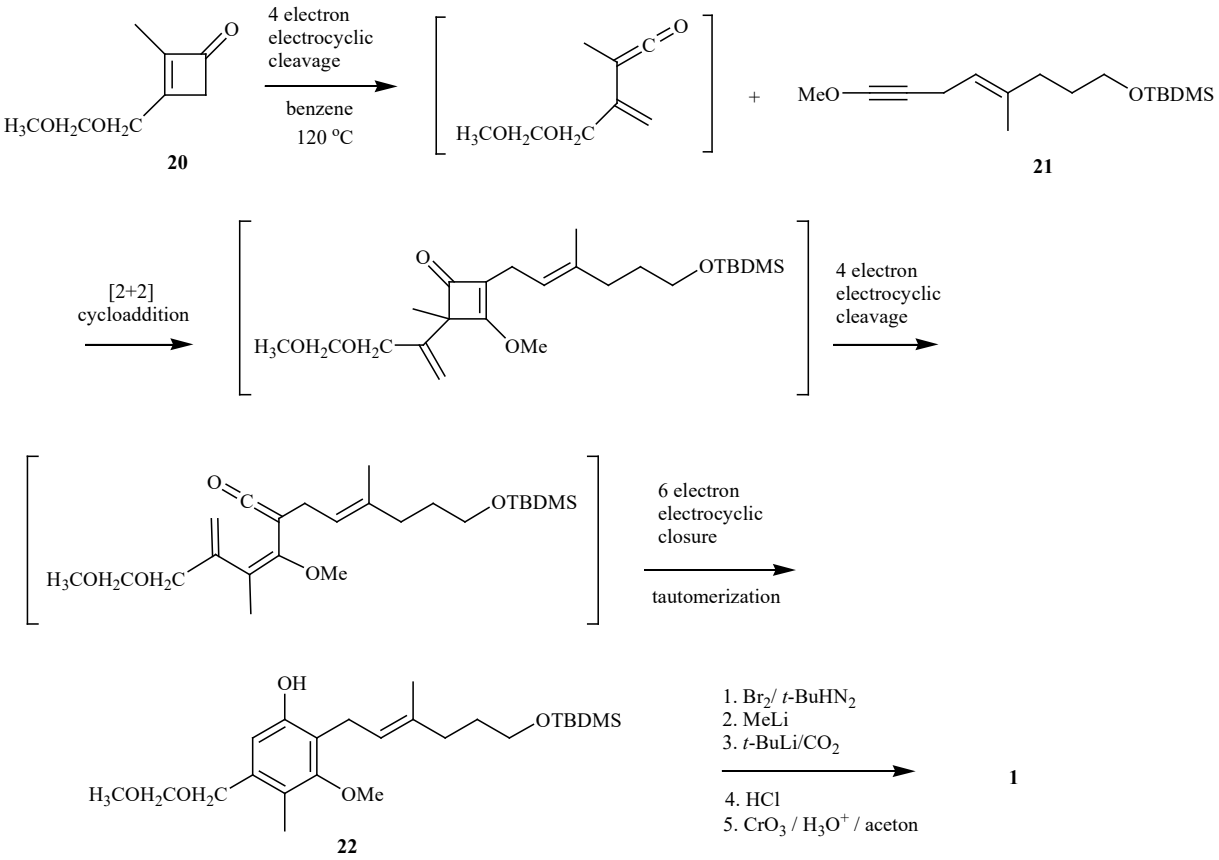
Scheme 2. Synthesis of mycophenolic acid **1** developed by Patterson [23, 24]

In the R.A. de la Cruz method [25] (Scheme **3**) allylic alcohol (**18**) was obtained in the different way than (**16**) (Scheme **2**). First, Diels-Alder cyclization and aromatization upon heating led to respective acetal (**19**). Next, recovered aldehyde bearing protected phenol group was treated with magnesium prop-2-enyl magnesium bromide.



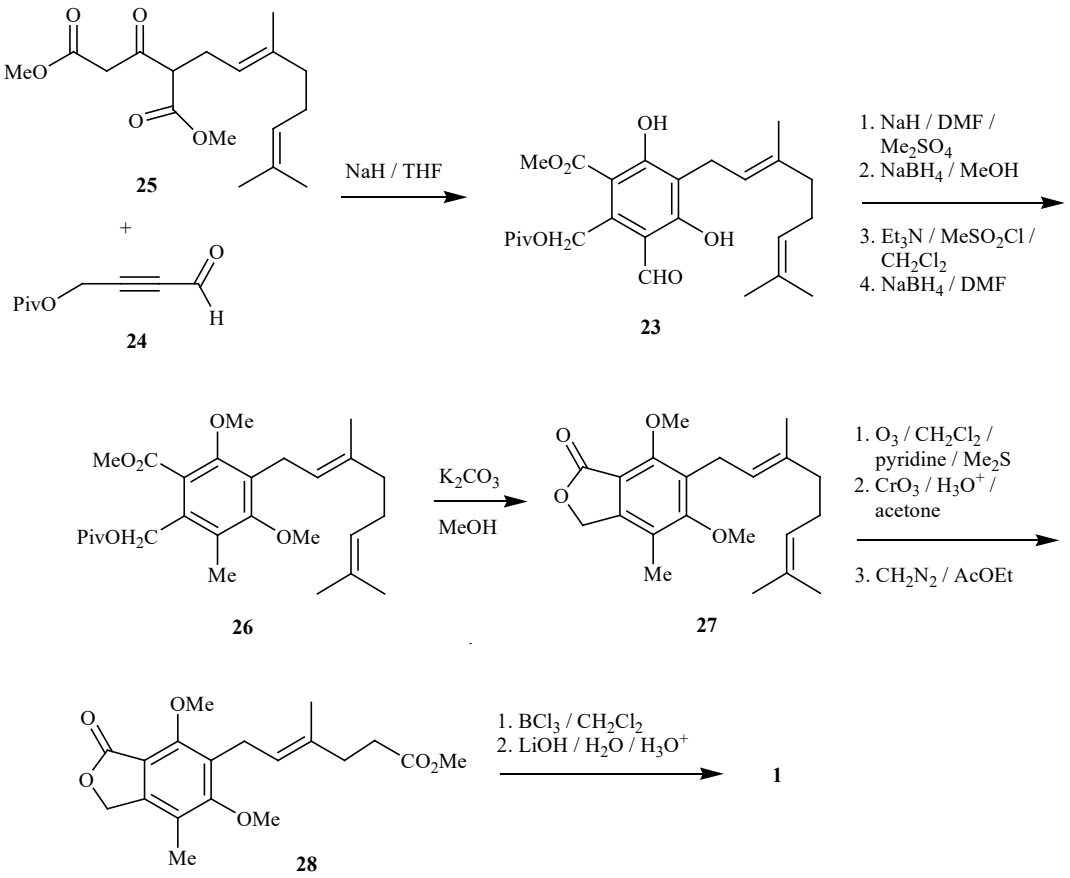
Scheme 3. Modification of MPA **1** synthesis proposed by R.A. de la Cruz [25]

An original method was proposed by Danheiser [27] (Scheme **4**). Cyclobutenone derivative (**20**) undergoes cycloaddition with alkenylated ether (**21**) via four-stages pericyclic reactions to five-substituted phenol (**22**), followed by ortho bromination, carboxylation and oxidation. Other cyclobutenones and alkenylated ethers are expected to give various analogues of MPA (**1**).



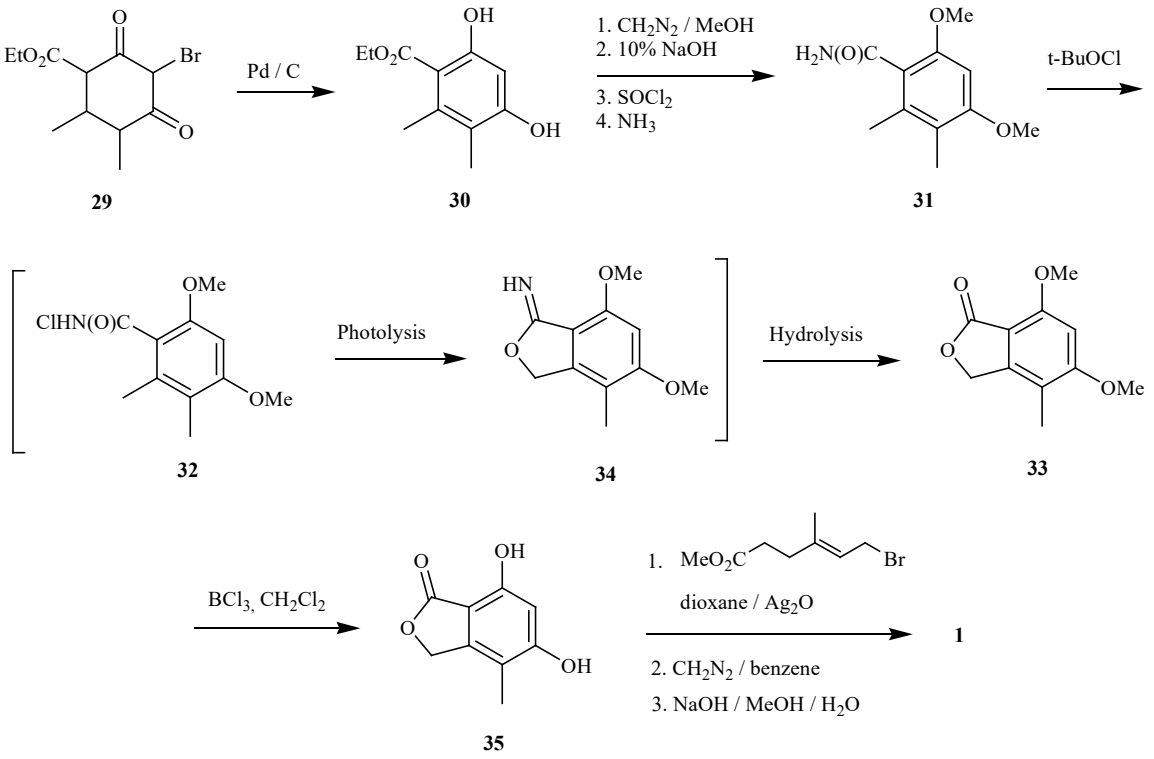
Scheme 4. MPA **1** synthesis according to Danheiser [27]

In the method reported by Covarrubiasa-Zuñiga (Scheme **5**) six-substituted benzene (**23**) is obtained in one stage [28-30]. Aldehyde (**24**) with geranyl derivative (**25**) gave Michael addition, followed by Dieckmann condensation. Then, phenol (**23**) groups were methylated and aldehyde group reducted to methyl. Subsequently, (**26**) was converted to lactone (**27**), which underwent two-stages oxidation and reaction with diazomethane to methyl ester (**28**). The ester (**28)** was selective demethylated and its hydrolysis gave MPA (**1**).



Scheme 5. The key stages in MPA **1** synthesis published by Covarrubiasa-Zuñiga [28-30]

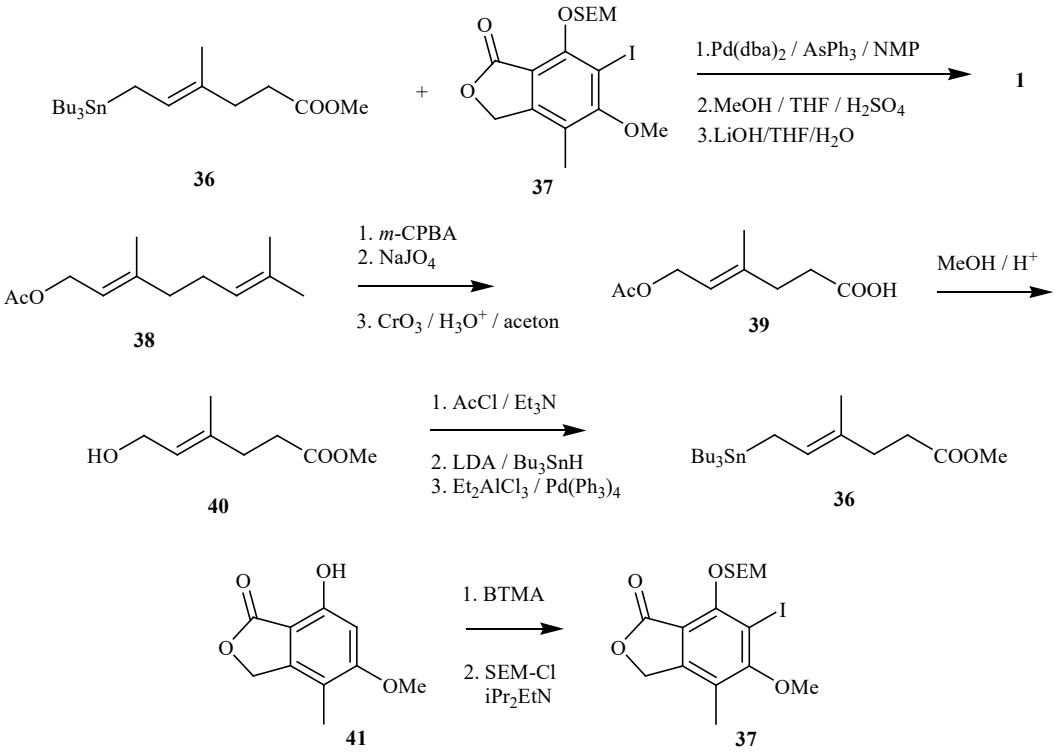
In the method developed by Canonica [26] (Scheme **6**) 1-carbethoxy-2,3-dimethylcyclohexa-4,6-dione (**29**) was aromatized on Pd/C catalyst to ethyl 4,6-dihydroxy-2,3-dimethylbenzoate (**30**). Then, the ester (**30)** was transformed into corresponding amide (**31**). Subsequently, the reaction with *t*-butyl hypochlorite provided N-chloroamide (**32**) as an intermediate, which upon photolysis and hydrolysis gave 5,7-dimethoxy-4-methylphtalide (**33**) via iminolactone (**34**). The lactone (**33**) was demethylated to (**35**), followed by reaction with methyl 6-bromo-4-methylhex-4-enoate. Finally, selective methylation and hydrolysis of the respective ester led to MPA (**1**).



Scheme 6. Synthetic pathway for MPA **1** according Canonica [26]

The other convergent approach to MPA synthesis has been proposed Plé [31] (Scheme **7**). The key step is coupling of methyl E-6-tributylstannyl-4-methyl-4-hexenoate (**36**) with methyl E-6-[1,3-dihydro-4-(2-(trimethylsilyl)ethoxymethoxy]-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl]-4-methyl-4-hexenoate (**37**). Deprotection of phenol group and hydrolysis of methyl ester produce MPA (**1**). Allyl tin (**36**) was obtained from geranyl acetate (**38**). First, it was oxidized via corresponding epoxide, aldehyde to E-6-acetoxy-4-methyl-4-hexenoic acid (**39**). Then, methyl E-6-hydroxy-4-methyl-4-hexenoate (**40**) was transformed to respective allyltin (**36**).

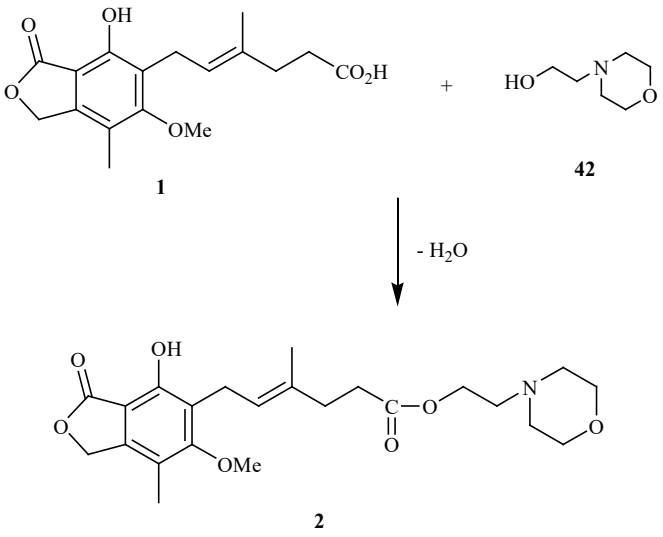
7-hydroxy-5-metoxy-4-methylphthalide (**41**) was obtained according to the procedure described by Canonica with slight modifications. Then, phthalide (**41**) was iodinated with benzyltrimethyl ammonium dichloroiodate (BTMA) and its phenol group was protected with 2-(trimethylsilyl)ethoxymethyl chloride.



Scheme 7. The convergent synthesis of MPA **1** described by Plé [31]

Synthesis of mycophenolate mofetil

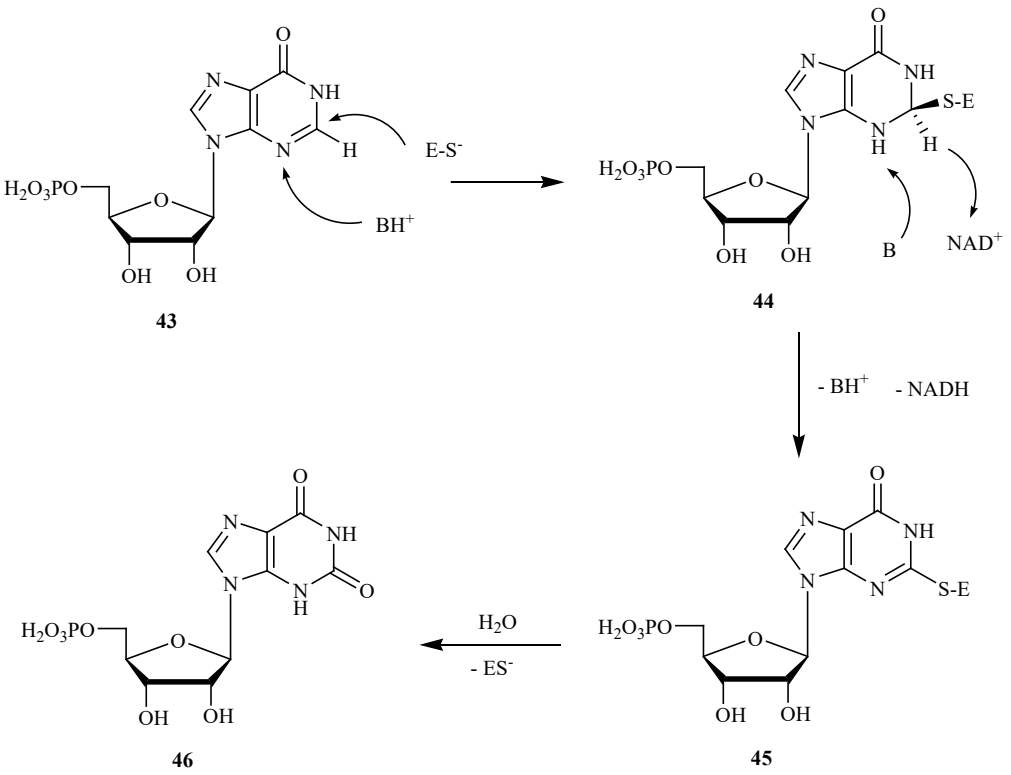
As mentioned previously, 2-morfolinoethyl ester – MMF (**2**) is a prodrug of MPA **1** widely used in clinical treatment. The reaction of MPA (**1**) with 2-morpholinoethanol (**42**) (Scheme **8**) can be performed in the presence of the coupling agent like DCC [34], substance capable to absorb water (inorganic salts, molecular sieves) [9] or in refluxed solvent, for instance xylene [38, 39]. The esterification can be also catalyzed by enzymes [40]. In an industrial scale unwanted color can be removed by addition of a substance which is able to form complexes with transition metals ions [9]. The small amounts of ethylenediamine, ethylenediamine tetraacetic acid (EDTA) can be add to the reaction mixture or during crystallization.



Scheme 8. Preparation of 2-morfolinoethyl ester - MMF **2**

Biological activity of mycophenolic acid. Mechanism of action

Mycophenolic acid (**1)** is an uncompetitive inhibitor of inosine-5'-monophosphate dehydrogenaze (IMPDH). This enzyme catalyzes biosynthesis of purine nucleotides which is crucial for lymphocytes proliferation (Scheme **9**). Since IMPDH is a target for immunosuppressive drugs, its structure as well as complexes with inosine-5'-monophosphate (IMP) (**43**) were determined. The reactive center in the enzyme molecule is a thiolate group $E\text{-}S^-$ from cysteine residue Cys 331. The purine ring of inosine-5'-monophosphate undergoes nucleophilic substitution at 2 position upon protonation, to form covalent bond with enzyme (**44**). Subsequently, hydride is transferred to NAD^+ and thioimidate intermediate (**45**) is hydrolyzed to xanthine-5'-monophosphate (XMP) (**46**). In this cycle, thioimidate intermediate (**45**) can be trapped by uncompetitive inhibitor as MPA [5].



Scheme 9. The process catalyzed by IMPDH

The structure of complex IMPDH – thioimidate intermediate (**45**) (Fig. (**2**) reveals numerous hydrogen bond interactions. Phosphate group contacts Gly 387, Gly 366, Ser 329 Ser 388, Tyr 411, hydroxyl groups of ribose unit Ser 68, Asp 364 and purine ring Met 414, Gly 415, Gln 441. Near to bound IMP is located MPA binding pocket. Interactions IMPDH – MPA (**1**) include hydrogen bonds of lactone moiety with Gly 326, Thr 333, phenol group with Thr 333, Gln 441, carboxylic group with Ser 276. Additionally, there are observed Van der Waals contacts of MPA molecule with Asn 303, Arg 322, Asp 274, Gly 415, Met 414 [5]. The structure investigations of IMPDH – MPA complex provide very significant implications within immunosuppressive properties of MPA (**1**). For example, presence of free phenolic group is crucial for biological activity. Similarly, *trans* configuration of double bond in side

chain enables formation of hydrogen bonds between carboxylic group and Ser 276 (amide hydrogen, hydroxyl group) [41].

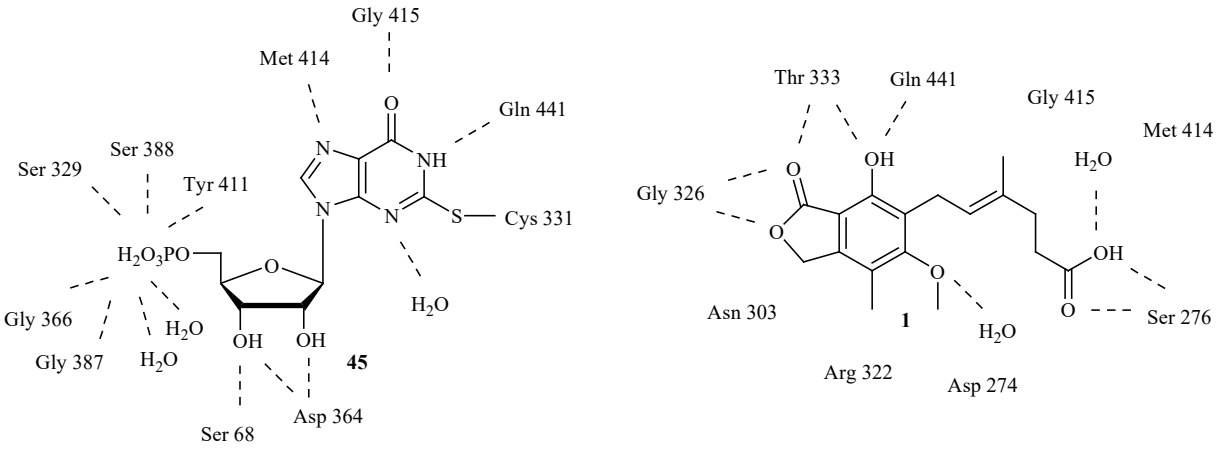
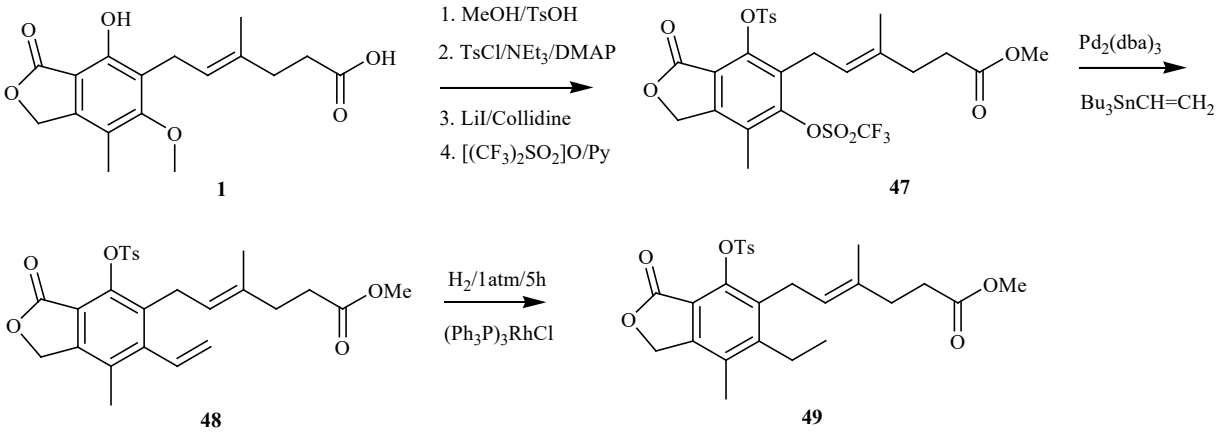


Fig. (2). Interactions of thioimidate analogue of xanthine-5'-monophosphate (XMP) **45** and MPA **1** with IMPDH

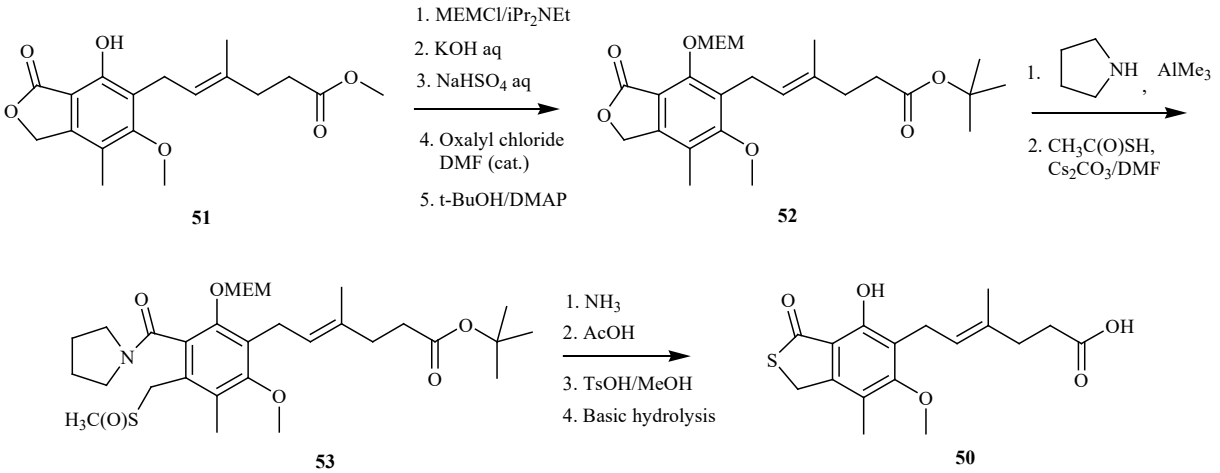
Synthesis of MPA derivatives

Among numerous synthesized MPA analogues only several exhibited higher *in vitro* or in *vivo* IMPDH inhibition in comparison with MPA (**1**). Their can be exampled by derivatives in which methoxyl group is replaced by vinyl or small alkyl substituent [16] (Scheme **10**). The starting material is MPA (**1**). The modification includes esterification with methanol, protection of the phenyl group, followed by conversion of methoxyl group to triflate ester (**47**). Then, coupling with tributylvinyltin gave vinyl derivative (**48**). This compound can be selectively hydrogenated to an ethyl analogue (**49**) [16].



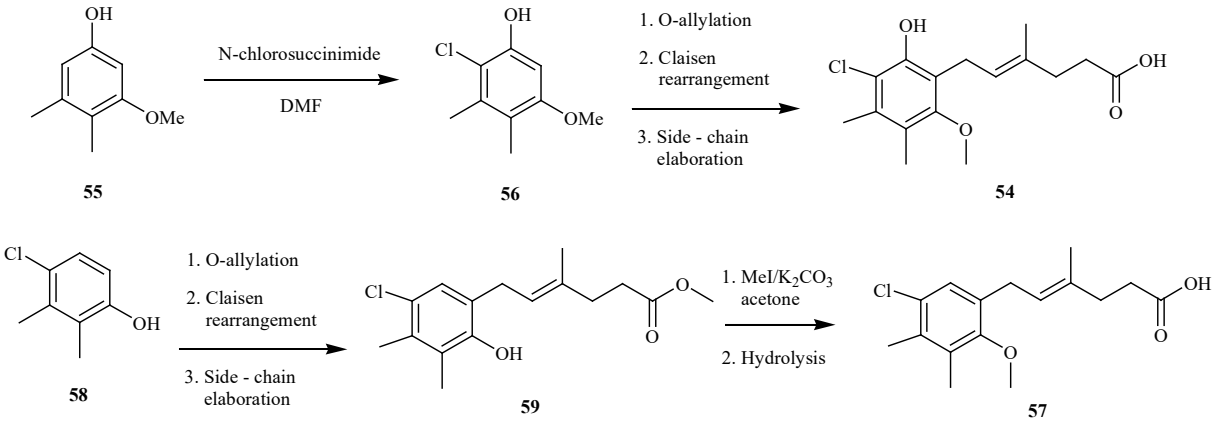
Scheme 10. Modification of MPA **1** structure with replacement of methoxyl substituent [16]

The all reported lactone ring modifications resulted in decreasing of potency. For instance, the thio-analogue of MPA (**50**) (Scheme **11**) indicated 5-fold lower activity [16]. Synthetic pathway requires lactone ring opening and starts from methyl mycophenolate (**51**), which was converted to *t*-butyl ester with phenol group protected as β-methoxyethoxymethyl ether (**52**). Subsequently, lactone ring in (**52**) was cleaved with aluminum amide (generated *in situ*), followed by reaction with thioloacetic acid. Thioacetate (**53**) underwent aminolysis and acidic cyclization to thiolactone. Deprotection of phenol group and basic hydrolysis of the adequate methyl ester produced desired thio-analogue (**50**) [16].



Scheme 11. Synthesis of thiolactone analogue of MPA **50** [16]

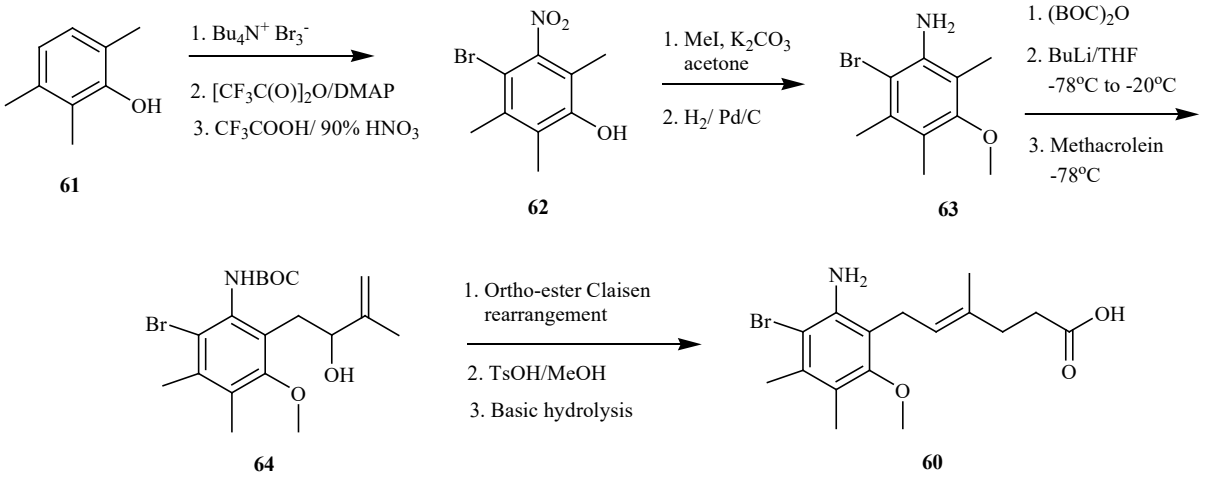
The other type of MPA analogues constitutes monocyclic phenols. Removing of lactone ring from the structure of MPA diminished biological activity considerably. However, the compound (**54**) (Scheme **12**) possessing chloride at ortho position according to phenol group gave relatively high IMPDH inhibition (about 70 % of MPA activity) [16]. Nelson and co-workers concluded, that monocyclic phenol – type derivative should bear a good hydrogen bond acceptor with a proper size. The chloride was introduced to 3-methoxy-4,5-dimethylphenol (**55**) in the reaction with N-chlorosuccinimide. Then, 2-chloro-5-metoxy-3,4-dimethylphenol (**56**) can be converted to MPA derivative (**54**) via number of stages described in the chemical literature (for instance see Patterson's synthetic route). The same research group established, that removing of hydroxyl group from such structures led to additional decrease of biological activity. Analogical non-phenol (**57**) occurred to be ca. 20-fold less potent than MPA (**1**). The reactions sequence starts from 4-chloro-2,3-dimethylphenol (**58**) via methyl (*E*)-6-(5-chloro-2-hydroxy-3,4-dimethylphenyl)-4-methyl-4-hexanoate (**59**) [16].



Scheme 12. Synthesis of monocyclic MPA analogues [16]

The replacement of hydroxyl group by amino group decreased biological activity similarly to non-phenols. The highest *in vitro* IMPDH inhibition for this type of amines was achieved for

(*E*)-6-(2-amino-3-bromo-6-methoxy-4,5-dimethylphenyl)-4-methyl-4-hexenoic acid (**60**) (Scheme **13**). It was about 20-fold less active than MPA (**1**). The synthesis of this kind of derivatives includes bromination with tetrabutylammonium tribromide followed by nitration of 2,3,6-trimethylphenol (**61**). Subsequently, 4-bromo-2,3,6-trimethyl-5-nitrophenol (**62**) was methylated and reduced to 6-bromo-3-methoxy-2,4,5-trimethylaniline (**63**). Then, ortho-methyl group was metalled using BuLi and treated with methacrolein to produce adequate allylic alcohol (**64**). Finally, ortho-ester Claisen rearrangement, deprotection of amine group and basic hydrolysis of the respective ester gave target derivative (**60**) [16].

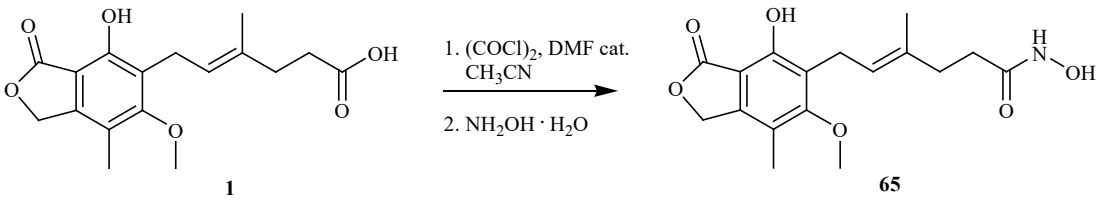


Scheme 13. Synthetic pathway towards amines derived from MPA [16]

Side – chain modifications

Mycophenolate mofetil MMF (**1**) is a MPA derivative used in clinic. An other example of side-chain modified MPA analogue is mycophenolic hydroxamic acid (MAHA) (**65**) [42] (Scheme **14**). This compound displayed comparable to MPA (**1**) IMPDH inhibition. An interesting properties MAHA (**65**) are also based on inhibition of histone deacetylases (HDECs), whereas MPA (**1**) occurred to be not active. These enzymes catalyze acetyl group

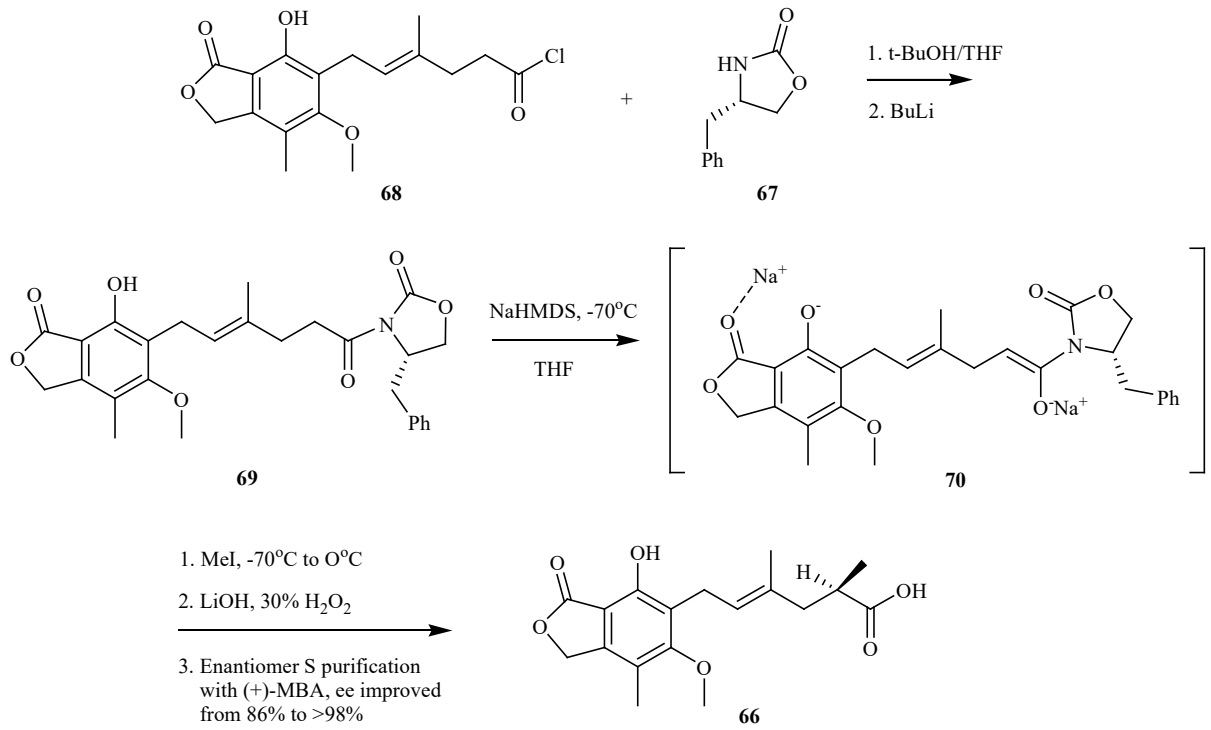
removal from lysine units in histones and are targets for anticancer drugs. MAHA (**65**) can be obtained by a short synthetic modification of MPA (**1**) [42].



1 **65**

Scheme 14. Synthesis of mycophenolic hydroxamic acid (MAHA) **65** [42]

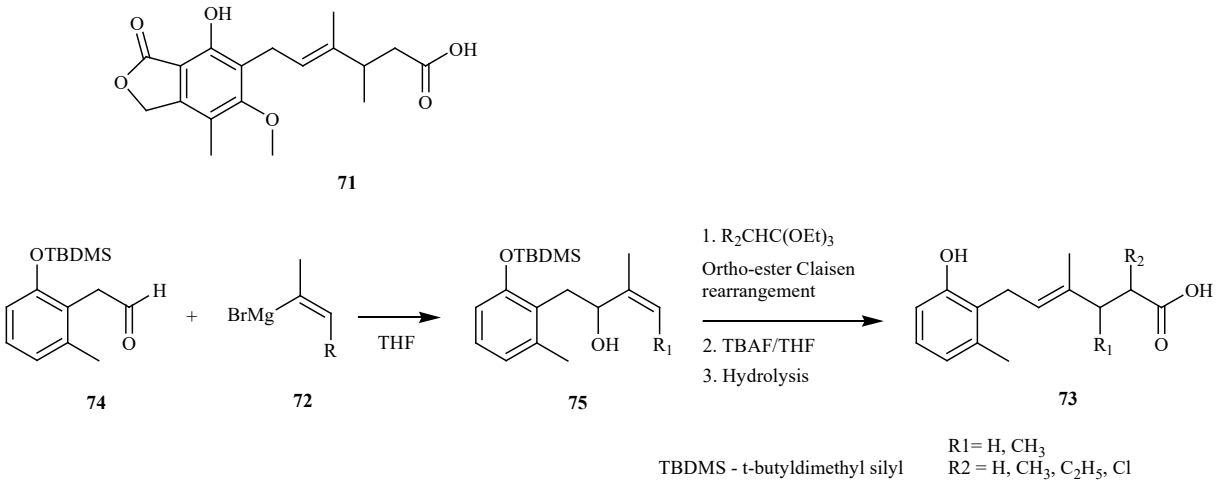
Rohloff and co-workers found that small alkyl groups at α position to the carboxylic group enhanced IMPDH inhibition *in vitro* [43]. There was mentioned in the communication that (S)-α-methylmycophenolic acid (**66**) (Scheme **15**) displayed ca. 5-fold higher activity than MPA (**1**). For synthesis of (S)-α-methylmycophenolic acid (**66**) authors used Evan's oxazolidinone methodology. The lithium salt of (S)-4-benzyl-2-oxazolidinone (**67**) was acylated with mycophenolic acid chloride (**68**) in the presence of generated *in situ* lithium tert-butoxide, and gave chiral imide (**69**). Then, reaction with hexamethyldisilylazide provides postulated dianion enolate (**70**). Presence of sodium phenolate protects benzyl position from deprotonation and prevents from numerous side - processes. Subsequently, enolate was C – methylated with MeI excess (5 eq), exclusively. Phenolic O-alkylation was also not observed because of tight chelation sodium counterion. The chiral auxiliary was removed from the structures of diastereoismeric imides with lithium hydroperoxide to give (S)-α-methylmycophenolic acid (**66**) (ee 86%). Optical purity was enhanced to ee >98 % by crystallization of α-methylmycophenolic acids (**66**) enantiomers as salts with (+)-α-methylbenzylamine ((+)-MBA) from acetone [43].



Scheme 15. Synthetic route towards (S)-α-methylmycophenolic acid **66** [43]

Oxazolidinone chiral auxiliaries were also used for preparation of four diastereoisomers of α,β-dimethylated mycophenolic acid from β-methylmycophenolic acid in high optical purity (ee > 99%, HPLC). No biological evaluations were published for these diastereoisomeric derivatives [44].

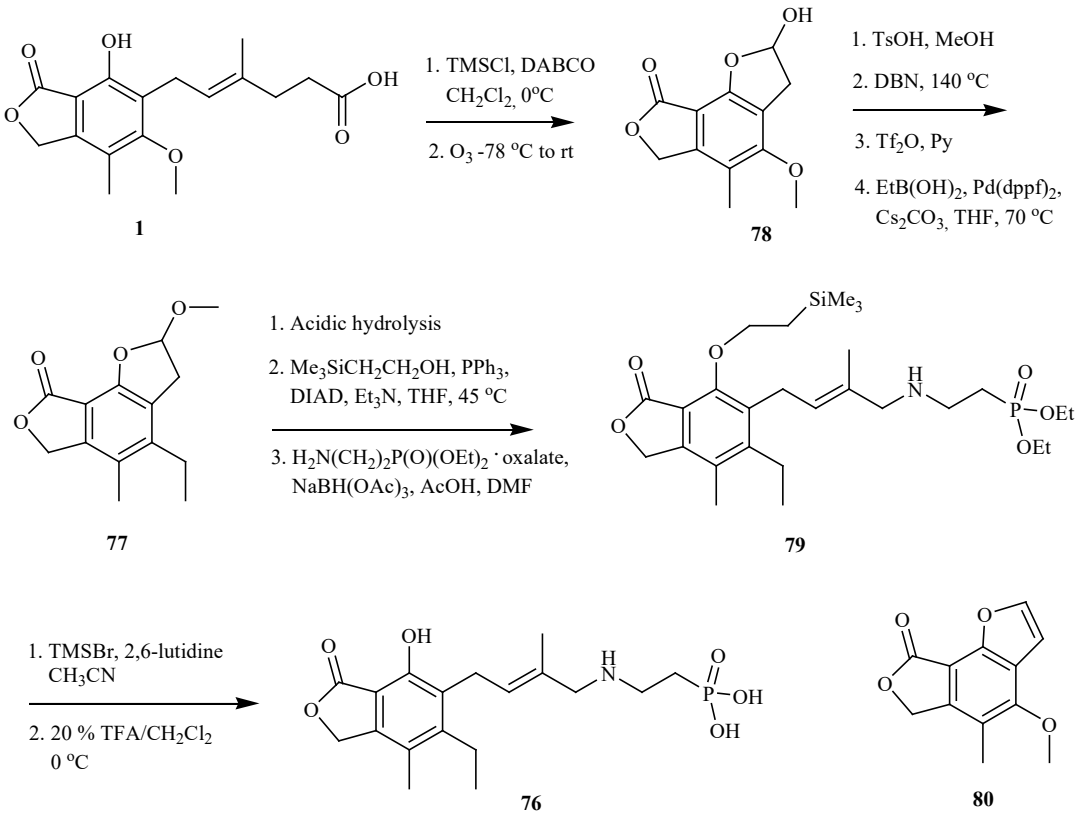
β-methylmycophenolic acids (**71**) (Scheme **16**) can be obtained upon structure modification of alkenyl magnesium bromide (**72**), like in case of synthesis of monocyclic derivative (**73**) from aldehydes (**74**) via allylic alcohols (**75**). None of the monocyclic analogs (**73**) occurred to be cytotoxic [45].



Scheme 16. Introduction of methyl group at β position in side – chain in MPA [45]

Watkins and co-workers described synthesis and biological examination of MPA derivatives possessing heteroatoms (O, NR with R = H, methyl, formyl, acetyl, sulfonyl) incorporated into side chain [46, 47]. Secondly, carboxylic group was replaced by phosphonic acid. In general, IMPDH inhibition was diminished but in some cases biological activity was maintained. Moreover, similarly to MPA (**1**), this type of compounds were more active towards IMPDH II. The highest inhibition, practically equal to MPA (**1**), was reached for β-aminophosphonic acid (**76**) (Scheme **17**) bearing ethyl group instead of methoxyl at C-6. In the semi-synthetic modification of MPA (**1**) respective acetal (**77**) is generated via ozonolysis, followed by methylation of hemiacetal (**78**). Then, demethylation of C-6 metoxyl group was achieved by heating in 1,5-diazabicyclo[4.3.0]non-5-ene (DBN). Subsequently, Suzuki coupling was used to replace of the respective triflate ester by ethyl group. Acetal ring was opened upon acidic hydrolysis and phenol group protected in Mitsunobu reaction with 2-(trimethylsilyl)ethanol in the presence of diisopropyl azodicarboxylate (DIAD). Protection of phenol group can be also performed with di-*tert*-butyl dicarbonate. Next, Wittig reaction followed by reduction led to O,O-diethyl β-aminophosphonate (**79**). Finally, deprotection

produced β-aminophosphonic acid [46, 47]. Noteworthy, benzofuran (**80**) can be used as a stable precursor for hemiacetal (**78**) [47].



Scheme 17. Synthesis of β-aminophosphonic MPA derivatives **76** with modified methoxyl substituent in aromatic ring [46-47]

MPA derivatives with adenosine moiety

Pankiewicz and co-workers developed MPA derivatives being resistant to glucuronidation *in vitro* under conditions where MPA was completely glucuronidated [48] Fig. (**3**). Therefore, mycophenolic adenine bis(phosphonate) analogues (**81-83**) are potential anticancer drug. Mycophenolic adenine dinucleotide (MAD) analogues (**81-83**) resemble the structure of nicotinamide adenine dinucleotide (NAD) which can bind to IMPDH. In contrast to NAD, MAD analogues (**81-83**) do not participate in hydride transfer and are able to inhibit the

enzyme. Moreover, they are more active towards IMPDH type II, that is the isoform observed at high levels in tumor cells and activated lymphocytes. When tumor cells are induced to differentiate, IMPDH II level decreases below the level of IMPDH I. As a result, it is expected that specific inhibition of IMPDH II may induce cellular differentiation and provide therapeutic efficiency together with elimination toxicity due to the inhibition of IMPDH I [48].

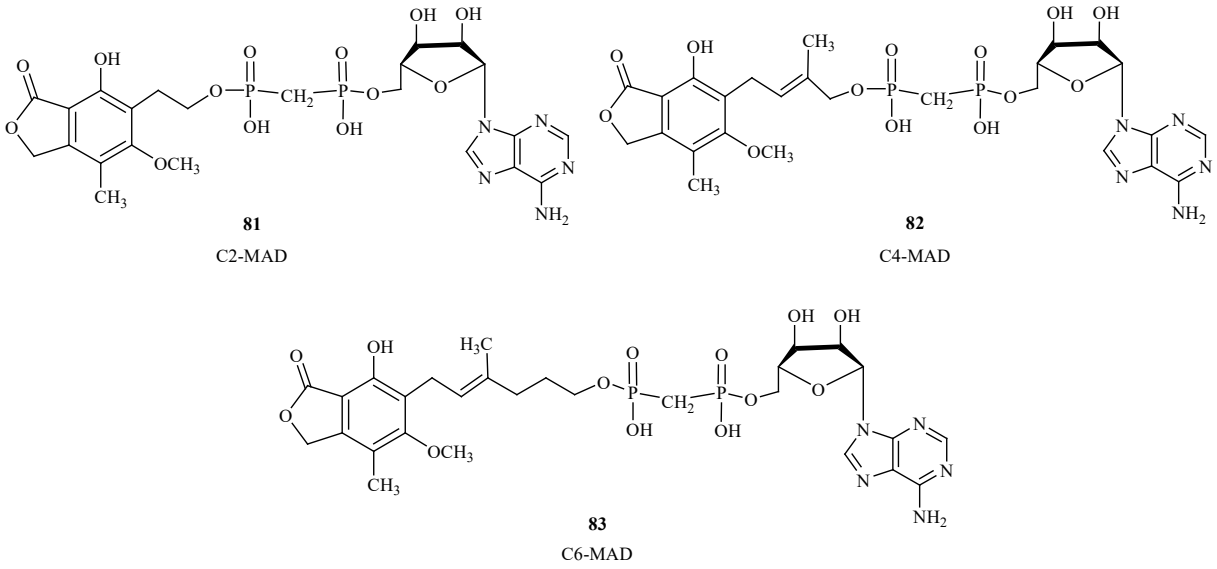
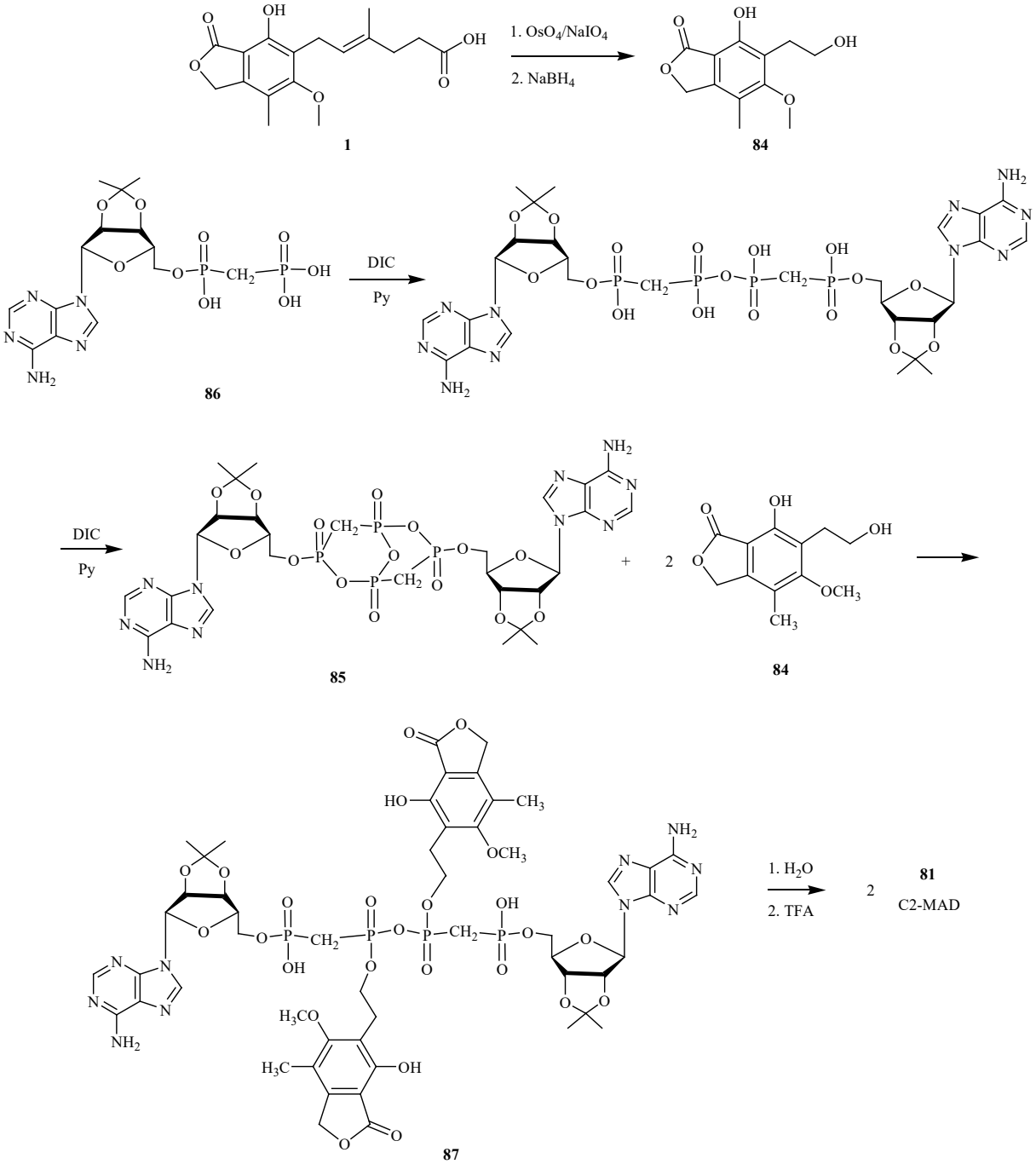


Fig. (3). Mycophenolic adenine dinucleotide (MAD) analogues **81-83** [48]

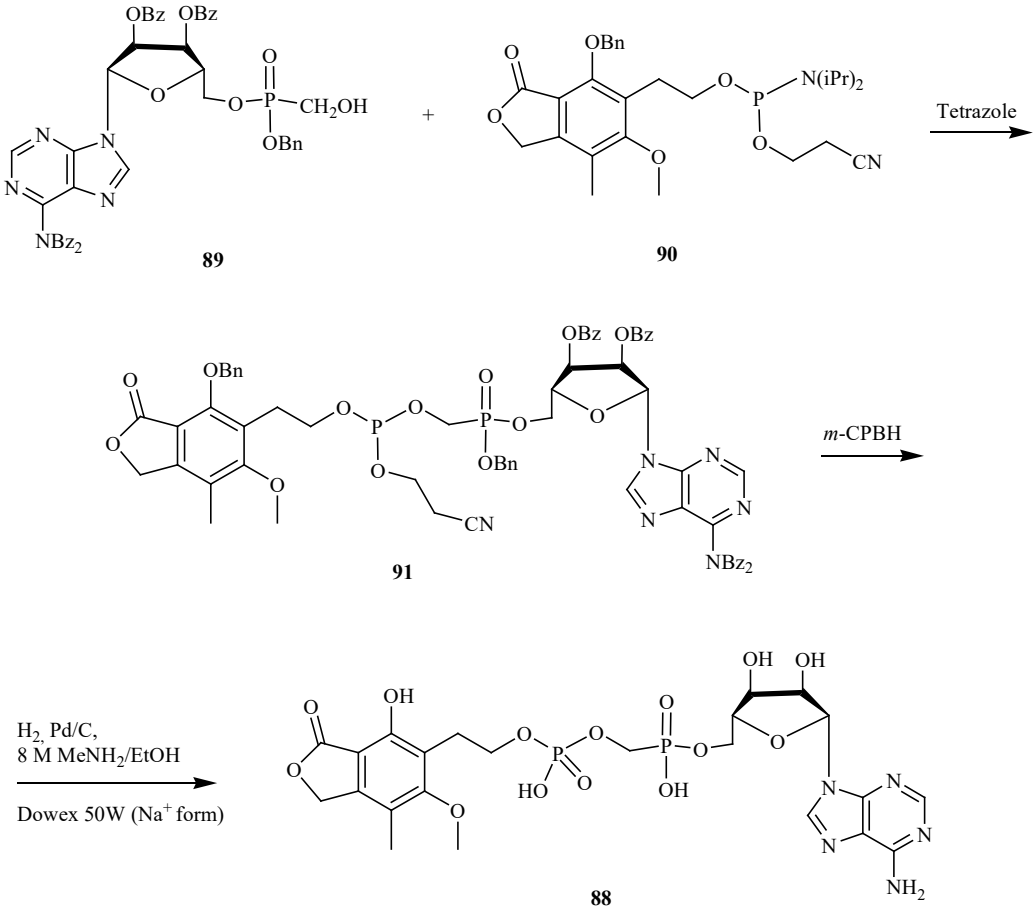
The structure of C2-MAD (**81**) includes two-carbon bridge between aromatic ring and phosphonate moiety (Scheme **18**). First, the double bond in the side chain of MPA (**1**) was oxidized followed by reduction of aldehyde to respective alcohol (**84**). The alcohol (**84**) reacts with bicyclic trisanhydride intermediate (**85**) derived from 2',3'-O-isopropylideneadenosine 5'-methylenebis(phosphonate) (**86**) upon treatment with diisopropylcarbodiimide (DIC) in dry pyridine. Then hydrolysis of diphosphonate ester (**87**) and deisopropylidenation with TFA gave C2-MAD (**81**).

Noteworthy, substitution of C2-MAD (**81**) at the adenine 2 position with ethyl or phenyl group improved its potency both as IMPDH inhibitor and against K562 cell proliferation [49]. In the similar manner were obtained C4-MAD (**82**) and C6-MAD (**83**) from adequate alcohols [48].



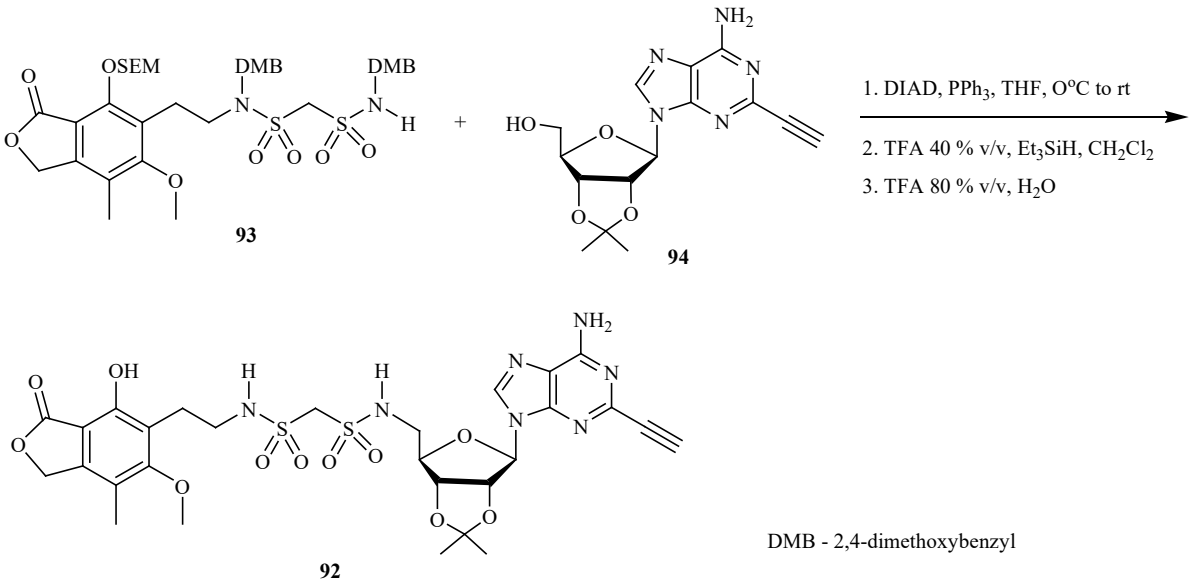
Scheme 18. Synthesis of C2-MAD **81** [48]

Other type of MPA analogues bearing phosphonate units are methylenephosphophosphonates (**88**) (Scheme **19**). These compounds revealed IMPDH inhibition near to MPA (**1**) [50]. Their ester bonds indicate good metabolic stability comparably to MAD (**81-83**) derivatives. The phosphitylation agents like 2-cyanoethyldiisopropyl-chlorophosphoroamidate can be used similarly to oligonucleotides synthesis. In this case 2',3'-*O*- dibenzoyl-N^6-dibenzoyl-5'-*O*-(hydroxymethyl)phosphonate (**89**) gives nucleophilic attack at trivalent phosphorus of 7-*O*-benzyl-C2-mycophenolic-cyanoethyl phosphoroamidate (**90**), followed by oxidation of (**91**) with *m*-CPBA. Then, deprotection of amine and hydroxyl groups leads to desired product (**88**) [50].



Scheme 19. The key step in synthesis of C2-MAD derivative **88** with phosphoric acid moiety [50]

Pankiewicz group obtained and evaluated also isosteric to MAD mycophenolic adenine bis(sulfonamide) MABS analogues [51] (Scheme **20**). Although their IMPDH inhibition was diminished, the activity was maintained and the best results gave ethynyl derivative (**92**). Mycophenolic acid (**1**) was converted to tri-substituted methylenebis(sulfonamide) (**93**) which underwent Mitsunobu coupling with 2',3'-isopropylidene-2-ethynyladenosine (**94**). Then, removing of SEM and DMB protective groups produces ethynyl MABS derivative (**92**) [51].

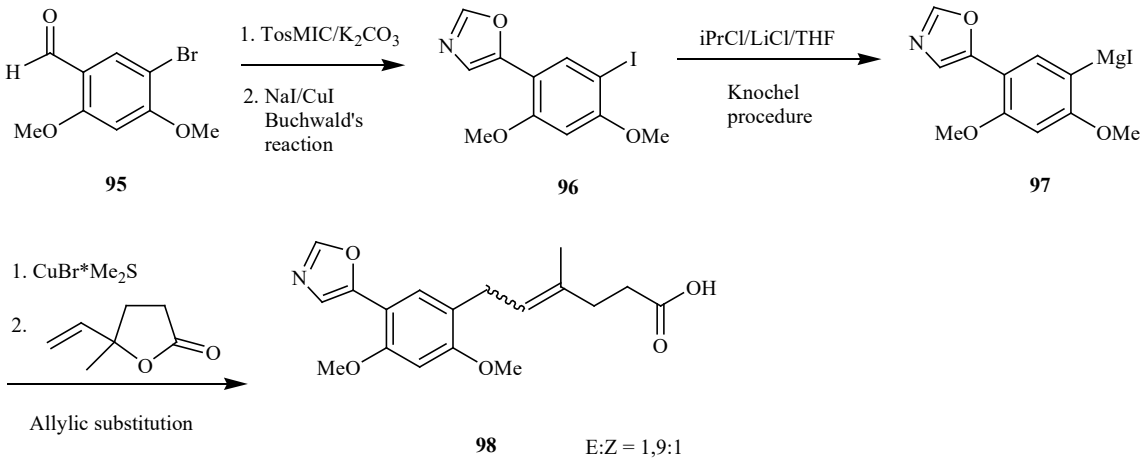


Scheme 20. Preparation of mycophenolic adenine bis(sulfonamides) MABS [51]

Heterocyclic MPA analogues

Glucuronidation of MPA (**1**) is a serious metabolic drawback of this compound. The C-7 phenolic group is believed to play significant role during glucuronidation processes. Pankiewicz developed oxazolyl derivatives (Scheme **21**) from the respective aldehyde (**95**)

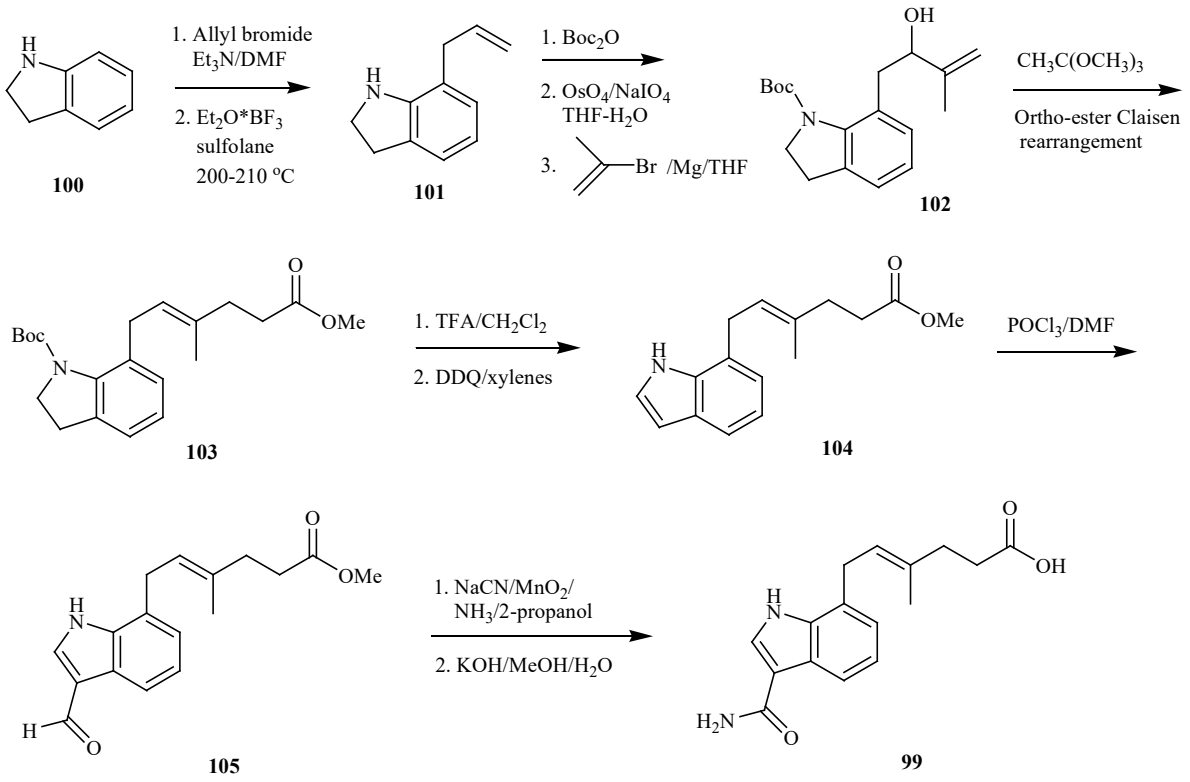
[11]. Heterocyclic ring was introduced in the dipolar cycloaddition with TosMIC (tosylmethyl isocyanide) followed by Buchwald's halogen exchange reaction to iodide (**96**). Subsequently, (**96**) was converted to appropriate cuprate via Grignard reagent (**97**), which gave allylic substitution with adequate lactone. The product (**98**) was separated to pure *E* and *Z* isomers. Both IMPDH I and II inhibition and K562 cell proliferation were lower if compared with MPA. Interestingly, double bond geometry did not influence biological activity [11].



Scheme 21. Synthesis of oxazolyl derivatives of MPA [11]

Anderson obtained indol MPA analogs (Scheme **22**) with hydrogen bonding N-H group instead of phenol one [17, 18]. The compound (**99**) was selected for advanced study against prostate cancer. The starting material was indoline (**100**). First, *N*-alkylation gave *N*-allylindoline, which underwent rearrangement to 7-allylindoline (**101**). Then, N-H indole group was protected with di-*tert*-butyl dicarbonate in dioxane and oxidation gave adequate aldehyde. The aldehyde reacted with 2-prop-2-enyl magnesium bromide to produce (**102**), followed by *ortho*-ester Claisen rearrangement. The Boc protecting group was removed from (**103**) and indoline derivative was dehydrogenated with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ). The Vilsmeier formylation of (**104**) provided aldehyde (**105**)

which was converted to respective amide. Subsequently, adequate methyl ester was hydrolyzed to desired product (**99**) [17, 18].



Scheme 22. Synthetic route to indol MPA analogues [17, 18]

Anderson research group developed also other MPA indole analogs according to molecular modeling concerning interactions with IMPDH, however anticancer activity have been not extended.

Conclusions, perspectives

Research area concerning extending of MPA use as a drug is still explored. Referring to number of scientific articles, biological and medical aspects are clearly predominant ones. However, synthesis of MPA analogs is also growing area and it can be anticipated that novel

drugs will be evaluated. The progress has been made also in view of drug delivery system. There was reported that MPA forms host-guest complexes with dendrimers like derived from poly(amidoamide) PAMAM. MPA is encapsulated strongly and such complexation depends on pH, dendrimer generation, ionic concentration. As a result, the release of the drug is influenced by presence of proteins [52].

The chemical behavior of MPA together with its promising biological activity is still being discovered and further progress in these studies can be expected.

Acknowledgement

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